TITLE OF THE INVENTION

Process for the Generation of Oligonucleotide Libraries (OLs) Representative of Genomes or Expressed mRNAs (cDNAs) and Uses Thereof

FIELD OF THE INVENTION

The present invention relates to a process for the generation oligonucleotide librairies (OLs) representative of genomes or expressed mRNAs (cDNAs) and to the uses thereof. In particular, the present invention relates to a process for the generation of oligonucleotide librairies comprising oligonucleotides of uniform length. The present invention further relates to the uses of these OLs in numerous biotechnological applications, including the identification and/or characterization of biological materials, clinical diagnosis (DNA/RNA level), preparative extraction of specific mRNA (and genes) and genomic research/mapping.

BACKGROUND OF THE INVENTION

The generation of genomic DNA libraries, or cDNA libraries and the maintenance, and handling of these libraries are critical procedures in the field of genomics and/or biotechnology. In classical libraries the relevant segments of DNA are cloned into vectors, which are maintained and propagated in particular biological systems (*in vivo*). Alternatively, libraries (*in vitro*) can be directly constructed from genomic DNA or cDNA. They contain linkers at the 5' and 3' ends of the DNA which allow PCR amplification of the library. The information stored in these libraries

contains repetitive sequence elements that originated from repetitive DNA, or high copy mRNAs. This results in a significant redundancy, which can complicate the use and the outcome of using classical libraries. Another important feature which reduces the utility of classical libraries is the heterogeneity in size of the members comprising the library. This limits the usefulness of classical libraries in subtractive hybridization procedures (1-2) which are dependent upon the length, complexity and the redundancy of the libraries, and which therefore are particularly sensitive to the choice of method and the number of cycles performed. In fact, one must tailor the hybridization conditions to accommodate the heterogeneous length and redundancy of stored information in order to perform subtraction. Thus, the results are more "laboratory-specific" than library-specific.

A number of diagnostic methods that involve nucleic acid hybridization have arisen in recent years. Most of them are designed to provide qualitative information about the presence of a specific sequence motif in a complex analytical mixture of nucleic acids and use a detection system based on PCR and/or DNA chip hybridization technologies (3-7). For both of these technologies, diagnostic oligonucleotides constitute an essential part of the detection system. These oligonucleotides are primarily chosen based on the sequence data of the nucleic acids to be detected. In spite of the power of hybridization to correctly identify a complementary strand, it does face limitations. In fact, the difference in stability between a perfectly matched complement and a complement mismatched at only one base can be as little as 0.5° C (8). This is the fundamental limitation to the power of DNA hybridization for specific identification of a cognate strand. Therefore, the diagnostic power of any chosen oligonucleotide must be validated using an analytical mixture whose sequence context is

not totally known. The problem of adequate probe selection is time and labour-consuming. On the other hand, the growing complexity of detection systems based on oligonucleotide technologies requires a fast selection of a large number of short oligonucleotides.

Akopyants *et al* (7) performed subtractive hybridization using bacterial DNAs digested by high-frequency restriction enzymes. The use of such restriction enzymes tends to generate DNA fragments having a broadly similar size, about 500 base pairs. However, the uniformity is not rigorous. Moreover, the library created by these restriction fragments still contains a significant number of redundant sequences; consequently, patches of short polymorphism embedded in homologous sequences are going to be missed when such a library is used.

U.S. Patent No. 5,270,163 (8) teaches a method for the isolation of nucleic acids using high-affinity nucleic acid ligands. This method has been termed the SELEX method (Systematic Evolution of Ligands by Experimental Enrichment) and is based on the use of proteins or small molecules, but not nucleic acids, as targets. The selection of oligonucleotides in the SELEX method relies on the three-dimensional (3D) shape of the oligonucleotides and their fit into the structures of the target molecules. In contrast to this, the selection of oligonucleotides in the present invention is based on hybridization with target nucleic acid.

Armour *et al* (11) describes the quantitative recovery of amplifiable probes hybridised to an immobilised target. The amplifiable probes consist of PCR or restriction fragments and their technique is meant to assess the copy number of *loci*.

There thus remains a need for oligonucleotide libraries which allow for the use of uniform hybridization conditions to perform selection and/or subtraction while minimizing or eliminating redundant sequences. Advantageously, these libraries can be used in the selection of highly informative and target-specific probe libraries. The present invention seeks to meet these and other needs.

SUMMARY OF THE INVENTION

The procedure described herein results in the generation and selection of oligonucleotide probes with a high specificity for a given system. These oligonucleotides cover the entire length of the target DNA, thus increasing detectability which might be lost in classical oligo-detection systems due to secondary DNA structure or DNA deletions present in an analyte mixture. At the same time, they present inexpensive variants of a multiplex oligonucleotide-detection approach, since they are not required to be individually synthesized.

More specifically, in accordance with the present invention, there is provided a process for the generation of oligonucleotide libraries, or OLs. The present invention teaches a process for generating OLs from genomic DNAs and cDNAs, and for performing the subtraction of these libraries.

The present invention further teaches OLs which allow the use of hybridization conditions which are controllable and reproducible. In addition, the invention teaches a process for the selection of uniform length OLs which minimizes or eliminates redundant sequences and reduces complexity. The result is the production of highly-informative and target-specific probe libraries.

An object of the present invention is therefore to provide a process for the generation of oligonucleotide libraries comprising OLs of uniform length which are self-amplifiable and easily subjected to subtraction.

Another object of the invention is to provide OLs which are compatible with DNA array technology. Indeed, an array of diverse mixtures of oligonucleotides which show differential hybridization patterns could be the best choice for the next generation of DNA diagnostics.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic representation of the experimental procedure for the preparation of OL. Denatured DNA is bound to the membrane and hybridized to the random oligonucleotides library in the presence of blockers. Theses blocking primers disallow the unspecific hybridization of left and right oligonucleotide arms used for PCR amplification of the OL. (ss - single-stranded DNA, ds - double-stranded DNA). Hybridization and PCR amplification of OL are described more particular below, in the Experimental Methods.

Figure 2: Dot blot hybridization of OL targeted against different genomes. The first row represents the dot blot hybridization of random probes with the specified genomic DNA (adenovirus, pBluescript and lambda). The

last row shows dot blot hybridization of mixed adenovirus and lambda-selected OL. The other rows are analytical dot blot hybridizations of selected OLs with each of the genomes indicated. The procedures of preparative and analytical hybridization are described in the Experimental Methods, below.

Figure 3: Specificity and probe distribution of OL generated from adenoviral genome. (**A**) The corresponding genome and adenoviral DNA were run on a 1% agarose gel stained with ethidium bromide. The type of restriction enzyme and DNA are indicated on the top of each gel lane. (**B**) Southern hybridization of the same gel using adenovirus OL as an hybridization probe (see Figure 2, row 2). It should be noted that under the experimental conditions, there was no cross-hybridization with either lambda or human DNA. (**C**) The same membrane was stripped and rehybridized with a OL directed against a 3648 bp-long restriction fragment. This subset of adenovirus OL was prepared by cutting the membrane corresponding to the 3648 bp band from a similar southern blot and reamplified by PCR as described in the Experimental Methods, below. Thus, it is shown that OL specificity may be enhanced by controlling the choice of targeted DNA fragments in the next round of selection.

Figure 4: The distribution of OL along genomic DNA. The densitrometric scan of radioactive signal from OL was integrated over total adenoviral genome (Figure 3, lanes 3 and 5) using Scion Image software (Scion corporation, Frederick, Maryland). The signal intensity of OL probes hybridizing to restriction fragments is linearly proportional to the length of DNA.

Figure 5: Subtractive enrichment of OL. (**A**) The tester OL is presented by the mixture of two genomes (Adenovirus type 2 and Lambda phage). The driver OL was produced from the Lambda genome only. The single stranded (ss) OL from driver DNA was used to pool out the complementary single stranded mixed tester OL. After removing the subtracted fraction, the remainder of the mixed OL was used as a probe in the analytical hybridization step. The mixed OL probes were analyzed by dot blotting as described (**B**) before subtractive enrichment and (**C**) after subtractive enrichment by hybridization to genomic Adenovirus and Lambda DNA.

Figure 6: Relative distribution of 20-mers with the different number of mismatches which do hybridize to targeted DNA. The abscissa shows the number of mismatches present in the 20-mer, while the y-axis illustrates the corresponding relative frequencies. The distribution profile was obtained by calculating the number of combinations for each particular number of mismatches which are thermostable at 52° C. The y-axis was normalized to reflect the relative distribution (%) over the total number of captured oligonucleotides (100%). The majority of n-mers captured after the first round of selection will be 20-mers with less then 6 mismatches. This is described further in the Detailed Description, below.

DETAILED DESCRIPTION

The present invention thus provides a process for the generation of oligonucleotide libraries having the following characteristics:

1) A uniform length of about 60 bases, comprising a central segment of about 20 bases randomly varied to represent all possible combinations,

and segments of about 20 bases of a defined sequence flanking the central segment on each side;

2) A uniform number of copies for each sequence motif (consequently, there are no differential hybridization kinetics which could originate from the presence of repetitive DNA); and

3) A melting profile which is characterized by a sharp transition from double stranded to single stranded (or vice versa) oligonucleotides. This is a critical advantage in subtractive hybridization procedures.

The use of these OLs enhances the specificity of hybridization to nucleic acids isolated from various sources, thereby allowing for the preparation of oligonucleotide mixtures useful in the detection and quantification of specific nucleic acids or nucleic acid mixtures.

In one particular embodiment, the starting pool of oligonucleotides is chemically synthesized and consists of a random region of a fixed length (L), flanked by a constant sequence (primer binding sites, PBS). The random oligonucleotide pool covers n copies (n=1,2,3...) of all sequence combinations of length L, i.e. 4^L, which is a total of 10^{12} different sequence motifs for L=20 nucleotides. The basic length of oligonucleotides is long enough to generate uniform sequence motifs for a particular biological system. The complexity of the library (10^{12}) overcomes the complexity of the template (which is usually between 10^4-10^9). The random pool is then hybridized with a nucleic acid template isolated from any selected source and the unbound oligonucleotides are washed away under stringent conditions. The remaining, template-bound oligonucleotides are then subjected to amplification, using PCR or other methods known to those of skill in the art and using primers complementary to the constant

flanking segments, thus producing a library of oligonucleotides capable of selectively hybridizing to nucleic acid templates.

The choice of 20-mer for the length of the oligonucleotide library is not arbitrary but is based on the rationale that the length of the particular sequence motif should be long enough to be unique for even the most complex genomes. The L-mer of length L will be, on average, repeated every 4 power L base pairs. The longer the L, the greater the average distance between 2 identical sequence motifs of length L will be. However, this particular combinatorial approach is at best approximative, and other lengths may be suitable as well. The choice of length will depend on such factors as the length and/or complexity of the genome to be detected and compatibility with current nucleic acid amplification and DNA array technologies.

In another embodiment, the amplification described above is performed with one of the PCR primers being labelled with biotin, providing means for purification of the labelled products with streptavidin-labelled substrates (12) or other similar methods. The amplified mixture of unlabelled oligonucleotides specific to one template is hybridized with labelled mixtures of oligonucleotides selected for specificity to one or several nucleic acid templates, and the unbound material is collected. In this manner, a mixture of nucleotides which is enriched for nucleic acids present in the unlabelled library only can be generated.

The process is based on stringent hybridization. Furthermore, high fidelity hybridization between pools of oligonucleotides and templates (genomic DNA or cDNA) is the basic mode of transfer of genomic

information into OLs. An efficient subtractive hybridization procedure is used to accommodate the features of the aforementioned OLs.

The present invention is illustrated in further detail by the following non-limiting example.

EXAMPLE 1

Generation of OLs, Use Thereof in Subtractive Hybridization to Generate Subtractive Oligonucleotides Libraries (SOLs), and Use of OLs or SOLs in Hybridization Experiments

EXPERIMENTAL METHODS

DNA / oligonucleotides

The starting random DNA pool was synthesised by GIBCO BRL (Burlington, Canada), (RAN), 5'-GCCTGTTGTGAGCCTCCTGTCGAA-N_{20}-TTGAGCGTTTATTCTTGTCTCCC-3'. The corresponding left and right arms were (LEFT) 5'-GCCTGTTGTGAGCCTCCTGTCGAA-3' and (RIGHT) 5'-BioGGGAGACAAGAATAAACGCTCAA-3'. The 5'-end biotinylated oligonucleotides were used to pool out complement strands, using BioMag magnetic particles (PerSeptive Biosystems, Framingham, MA). During preparative hybridization, the left and right arms were blocked by (LEFT) 5'-TTCGACAGGAGGCTCACAACAGGC-3' and (RIGHT) 5'GGGAGACAAGAATAAACGCTCAA-3'. Theses oligonucleotides are termed 'blockers' in the text.

The following genomic DNA was used to produce OL: Adenovirus DNA Type 2, (GIBCO BRL), Lambda DNA cI857 *ind1 Sam 7* (New England Biolabs), pBluescript II SK(+) (Stratagene, San Diego, CA). The Human HeLa DNA used as one control was from Clontech (Palo Alto, CA).

Blotting genomic DNA

The genomic DNA was denatured 2-3 minutes at 95°C and cooled on ice. The nylon membrane (Hybond-N, Amersham Pharmacia Biotech, Piscataway, NJ) was blotted with 100 ng of denatured genomic DNA, dried for 2 minutes on a hot plate and exposed to UV light for 8 minutes. The prehybridization was done for a minimum of 30 minutes in the hybridization buffer (7% SDS, 0.25M Na2HPO4 pH7.4, 1mM EDTA, pH 8.0 and 10g/L of BSA).

Hybridization and washing of the starting random pool

The preparative hybridization between random core (20N) and targeted DNA was done with 10 pmoles of starting random pool (RAN). The random pool was pre-mixed with 100 pmoles (10 times more than RAN) of LEFT and RIGHT blockers in order to exclude cross-hybridization of left and right arms with genomic DNA. The oligonucleotide mixture was heated up to 95°C, cooled at room temperature and added to the hybridization buffer. The hybridization was done overnight at 50° C. The first washing was done with 6X SSC, followed by subsequent 2X SSC washing at the same temperature as hybridization was done.

Generating OL by PCR

The dot containing the genomic DNA and bound probes was cut out of the nylon membrane (radius of 2-4mm), soaked in 100 µl H_20 and heated to 95°C for 1-2 minutes. The solution containing the denatured probe (originally RAN) was then collected and passed threw a Sephadex G-50 column in order to eliminate salts and SDS. The PCR was prepared under standard conditions, typical for SELEX-like amplification of DNA (10, 13). The RIGHT 5'-end biotinylated primer of the sense strand (the

one which did not hybridise with genomic DNA) and LEFT primer of antisense strand were used in the PCR reaction. The temperature cycles were 53°C, 72°C, 95°C, each 30 seconds, repeated 20 times.

Probe labelling and hybridization

Before labelling, the PCR reaction mixtures were passed threw Sephadex G-50 columns. Around 100-200 ng of PCR product was labelled with 50 pmols of γP^{32} ATP (6000 Ci/mmol, I.C.N. pharmaceuticals, Irvine, CA). The total amount of probe radioactivity was 300 000 c.p.m. The probe was added into 0.5 ml of hybridization buffer. The blotting of genomic DNA was done as described above. Hybridizations were done overnight at 50°C. The nylon membrane was washed as previously described, and exposed to Kodak X-OMAT film.

OL labelling and analytical hybridization

The generated OL was tested, using 1) the original genomic DNA from which they were selected (positive control) and 2) using the unrelated genomic DNA (negative control). The OL labelling, hybridization and probe washing was done as described, except that hybridization time was shorter (60 minutes).

Southern blot hybridization

Electrophoresis was performed in a 1% agarose gel with TBE buffer (80 mM Tris borate, pH 8.0, 2mM Na_2EDTA) and stained with ethidium bromide. One ug of BstEII-digested lambda DNA, 300 ng of adenoviral DNA and 1 µg of AluI-HpaI-digested human HeLa DNA were run on the gel according to specifications (all restriction enzymes used in this work were purchased from New England Biolabs). For Southern hybridization, DNA was transferred to Nylon membranes by capillary blot procedure

following manufacturer's recommendations (Amersham Pharmacia Biotech). Hybridization was performed as described above with adenoviral OL. Autoradiographic exposure (using Kodak X-OMAT film) was done at room temperature, for few hours. Stripping of the membrane was done by boiling a 1% SDS solution and pouring it over the Nylon membrane.

Subtractive enrichment of OL

The tester OL (mixed OL) that reflects the two genomes (Adenovirus type 2 and Lambda) was made by preparing OL from equimolar mixtures of 2 genomes. The driver OL was produced from the lambda genome only. The production of sense strand (the one which did not hybridize with genomic DNA) was done using 5'-end biotinylated primer in PCR reaction. After denaturing PCR product, the biotinylated sense strand was bound to streptavidin magnetic particles (200 µg, binding capacity > 200 pmols of biotinylated oligonucleotides, Biomag Magnetic Particles, PerSeptive Biosystems), and pulled-out using a magnet. The complementary antisense strand was discarded with the liquid phase. The mixed antisense tester OL (Lambda + Adenovirus DNA) was produced in the same way. This time, the supernatant with the antisense, non-biotinylated strand was hybridized overnight at 50°C with 10 times molar excess of driver Lambda sense stand attached to magnetic beads. The hybridization buffer was the same as described above but without SDS. After removing the fraction bound to the magnetic beads, the rest of the mixture was used in the analytical hybridization step.

RESULTS

The starting random pool of oligonucleotides contains 4^{20} (i.e. 10^{12}) different 20-mers. The diversity of the sequence motifs is approximately

10^{11} higher than the diversity of the most complex genomes. A schematic representation of the procedure for generating OL is presented in Figure 1 and is described in detail in the Experimental Methods, above.

Blockers were used in order to avoid hybridization of the flanking arms to the targeted genome, and this step was found to be critical to achieve specificity. The stringency of hybridization conditions eliminates unbound 20-mers, leaving the specific oligonucleotides bound to the membrane via hybridization of the random core to the genome (Fig. 1). This ensemble of selected oligonucleotides constitutes the OL.

It should be noted that the starting random pool of oligonucleotides contained about 8 copies of each sequence motif during the first hybridization step (10-20 pmoles) and that the number of copies of each particular 20-mer present in the random mixture was smaller than the number of genome copies.

Figure 2 shows that OLs are able to discriminate genomes with complexities around 10^3 to 10^4. The starting random pool of probes binds to all three genomes equally (Fig. 2, row 1). After one round of selection, the OL can hybridise specifically towards a single targeted genome (Fig. 2, rows 2, 3 and 4). The OL can be selected against a mixture of two genomes and the specificity is conserved for both genomes (Fig. 2, row 5).

A Southern blot was performed in order to document the distribution of adenovirus OL probes along the genome (Fig. 3). There was no apparent cross-hybridization of adenovirus OL to either HeLa or Lambda DNA (Fig. 3b, lanes 1, 4, 5 and 6). The intensity of radioactive signal over adenoviral genome generated by adenovirus-specific OL was linearly increasing with

the DNA fragments' length (Fig. 4). Therefore, one could deduce a uniform distribution of OL throughout the genomic DNA.

In the next step, only the subset of the adenovirus OL bound to the 3648 bp band in Figure 3b (rows 3 and 6) was reamplified and selected. The membrane was washed from the original probe and hybridised with the 3648 bp subset of the original OL. Figure 3c shows that the specificity of the OL subset is obtained against the 3648 bp band. These data illustrate the successful increase in the specificity and the reduction in the complexity of the original Adeno-specific OL to that of the 3648 bp subset, using just one additional round of selection.

One round of subtractive enrichment between two oligonucleotide libraries was performed as schematized (Fig. 5a). The tester OL reflects the two genomes (adenovirus type 2 and lambda phage). The driver OL was produced from the Lambda genome only. The single stranded (ss) OL from the driver DNA was used to pool out the complementary single stranded, mixed, tester OL. After removing the subtracted fraction, the rest of ssDNA was used as a probe in the analytical hybridization step. The intensity of hybridization signals between Lambda and Adeno genomes, before (Fig. 5b) and after (Figure 5c) one round of subtractive enrichment was shown. It should be noted that further subtraction steps could be performed by changing the sequence design of flanking arms between tester and driver OLs, as suggested by recent developments in subtractive procedures (14).

With reference to Figure 6, the relative distribution of 20-mers with different numbers of mismatches that hybridized to the targeted DNA was predicted. The number of 20-mers (N) with (m) number of mismatches

(m=0, 1, ..., 20) capable of hybridizing to the target sequence were then calculated. First, the number of combinations of 20-mers (C) with the same number of mismatches (m) in the initial random pool of oligonucleotides that are capable of hybridizing to a specific 20-mer motif was calculated. Since each full match could be replaced by 3 different mismatches, the number of combinations must be multiplied by 3^m i.e. $C*3^m$. Finally, these numbers were adjusted to reflect the sequence-dependent termostability of 20-mers with (m =0, 1...20) number of mismatches which hybridised at 52°C using the thermostable fraction of the binomial distribution for each n-mer population. Duplexes of more than 7 mismatches are not observed based on this themostability criterion. Therefore, the majority of 20-mers captured after the first round of selection will harbour less than 6 mismatches.

A process that generates amplifiable DNA oligonucleotide libraries which are specific for a given segment of DNA has been described. This process is akin to random priming, because it is possible to generate probes without *a priori* knowledge of the template sequence. One round of preparative hybridization was enough to produce genome-specific oligonucleotide libraries (Figs. 2 and 3). The OLs were inferred from genomes of complexity of 10^3-10^4.

The process described herein generates probes with high detection power. These probes/selected oligonucleotides can contain mismatches. The notion that introduction of artificial mismatches could increase detection power of oligonucleotides during single nucleotide polymorphism (SNP) detection was well documented by Guo *et al* (6). However, the prediction of positions and types of mismatches, which should be introduced to increase detectability of oligonucleotide, remains

undefined. Consequently, to enhance oligonucleotide detectability by introducing (artificial) mismatches, one must search different positions and types of mismatches along the oligonucletide. Once they are empirically determined, i.e. tested on 2 different sequence motifs, the oligonucleotide containing particular mismatches could be used (15). The present process provides an approach based on differential selection of thermostable oligonucleotides (i.e. their differential stability), which are present in one, but not in the second system. The selection of oligonucleotides with the highest detectability is inherently present in this process, i.e. the method suggests a solution to the problem of where and what type of mismatches should be introduced to increase detection power of oligonucleotide, or to find the particular oligonucleotide which best discriminates between 2 sequence motifs which may differ by a single base.

Without wishing to be bound by any hypothesis, the following provides an explanation of what is believed to be occurring during the process of the present invention. Based on calculations, it is expected that the 20-mers selected in an OL can contain up to 6 mismatches. Nevertheless, specificity toward a given template was achieved, suggesting that the presence of these putative mismatches did not interfere with good discrimination. In other words, mismatch-free hybridization is not critical for differential detection approach; rather, the *relative differences* in the thermodynamical stabilities of the hybridized oligonucleotides appear to be determinative. The present process uses selection of oligonucleotides based on this criterion and therefore provides the possibility of overcoming current technological limitations. In the second and further rounds of selection, the number of 20-mers both in the targeted genome and the probe mixture (OL) could be adjusted. Each new round of

preparative hybridization (Fig.1) and/or subtraction (Fig. 6) could reduce the complexity of OL, by using the excess OL rather than genomic DNA. Therefore, the average number of mismatches for each particular 20-mer will continue to decline until it reaches the sequence-dependent limitation, but not the concentration-dependent limitation.

In summary, OLs are generated from the template DNAs. These OLs are used in subtractive hybridization, for example between genomic or cDNA-based libraries (OL1 and OL2) to make a new Subtractive Oligonucleotide Library (SOL1/2 and/or SOL2/1)), that is/are specific for one system/library but not for the other. Oligonucleotides isolated from such subtractive libraries (SOL) are useful for diagnostic purposes. They can a) directly serve as highly specific hybridization probes or b) they can be tested for PCR-specific differential amplification, specific for one, but not the other biological system.

These libraries (OL or SOL) can be hybridized to oligonucleotide chip arrays in order to obtain a specific hybridization pattern that is useful for diagnostic features: each OL produces an image which is specific for the templated DNA (genome or cDNA). A particular advantage in using OL or SOL instead of genomic/cDNA libraries is that the hybridization signal is not dependent on copy number and distribution of particular sequence motifs. By comparing images of different genomes/cDNA, one can deduce which oligonucleotides are highly specific for a single genome/cDNA, and use this or these oligonucleotide(s) as "genome tags". The oligonucleotides obtained can also be used for specific diagnostic PCR.

OLs or SOLs can be inferred from two biologically relevant systems, like mammalian cells, to detect fine differences in cell cycle, tissue status, viral infection, age/development status etc.

Although the present invention has been described hereinabove by way of a preferred embodiment, it can be modified by one of skill in the art without departing from the spirit and nature of the subject invention, as defined more particularly in the appended claims.

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